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Plasma Levels of Soluble E-Selectin in Patients With Disseminated Intravascular Coagulation

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The plasma level of soluble E-selectin (sE) reflects the activation of endothelial cells induced by cytokines such as tumor necrosis factor-α and interleukin-1 in vitro. These cytokines are important in the development of coagulation abnormalities in patients with sepsis. We compared the plasma levels of sE in patients with infections suspected of having disseminated intravascular coagulation (DIC) (n = 33) and in patients with underlying disorders other than infections, including solid tumors (n = 28), obstetric disorders (n = 13), hematologic malignancies (n = 13), and liver disease (n = 9), to clarify the involvement of cytokines in the development of coagulation abnormalities in patients with sepsis. Plasma levels of sE in patients with infection were significantly higher than in patients with the other underlying disorders. The plasma level of sE was also significantly higher in patients with infection with DIC (114.6 ± 77.9 ng/ml, n = 21) than in patients with infection without DIC (54.5 ± 53.1 ng/ml, n = 12, $P < 0.02$). There was no significant difference in sE level between patients with the other underlying disorders with and without DIC. The plasma level of sE was significantly correlated with the serum level of FDP(E) in patients with infection. The plasma level of sE was significantly higher in patients with infection with organ failure compared to patients without organ failure. There was no significant difference between patients with the other underlying disorders with and without organ failure. Plasma levels of tumor necrosis factor-α and interleukin-6 were detected in only 12.1% and 20.0% of patients with infections, respectively. These observations strongly suggest that plasma levels of sE reflect the activation of endothelial cells induced by cytokines, which may lead to DIC and organ failure in the presence of sepsis. Furthermore, determination of plasma level of sE may be useful for detecting the endothelial activation induced by cytokines in the pathologic conditions of sepsis, even when plasma levels of cytokines cannot be detected. Am. J. Hematol. 54:219–224, 1997 © 1997 Wiley-Liss, Inc.

Key words: soluble E-selectin; cytokines; disseminated intravascular coagulation; sepsis; plasma levels

INTRODUCTION

Cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are important in the pathogenesis of sepsis by activating endothelial cells as well as leukocytes [1]. These cytokines increase expression of tissue factor, which activates the extrinsic coagulation pathway [2,3] on endothelial cell surfaces, and reduce the endothelial antithrombogenic potential, by reducing thrombomodulin activity [4] and glycosaminoglycan content [5]. They also increase endothelial expression of E-selectin, an endothelial leukocyte adhesion molecule that enables activated neutrophils to adhere to endothelial cells [6]. The E-selectin-mediated contact between activated neutrophils and endothelial cells is a critical factor in neutrophil-induced endothelial cell injury [7]. Thus, TNF-α and IL-1β may be important in both coagulation abnormalities and organ damage in patients with sepsis.

Soluble E-selectin (sE) is present in the supernatants of cultured endothelial cells activated by interleukin-1β, and the serum level of sE is elevated in patients with septic shock [8]. Thus, it is possible that serum levels of sE may reflect cytokine-induced endothelial cell activa-

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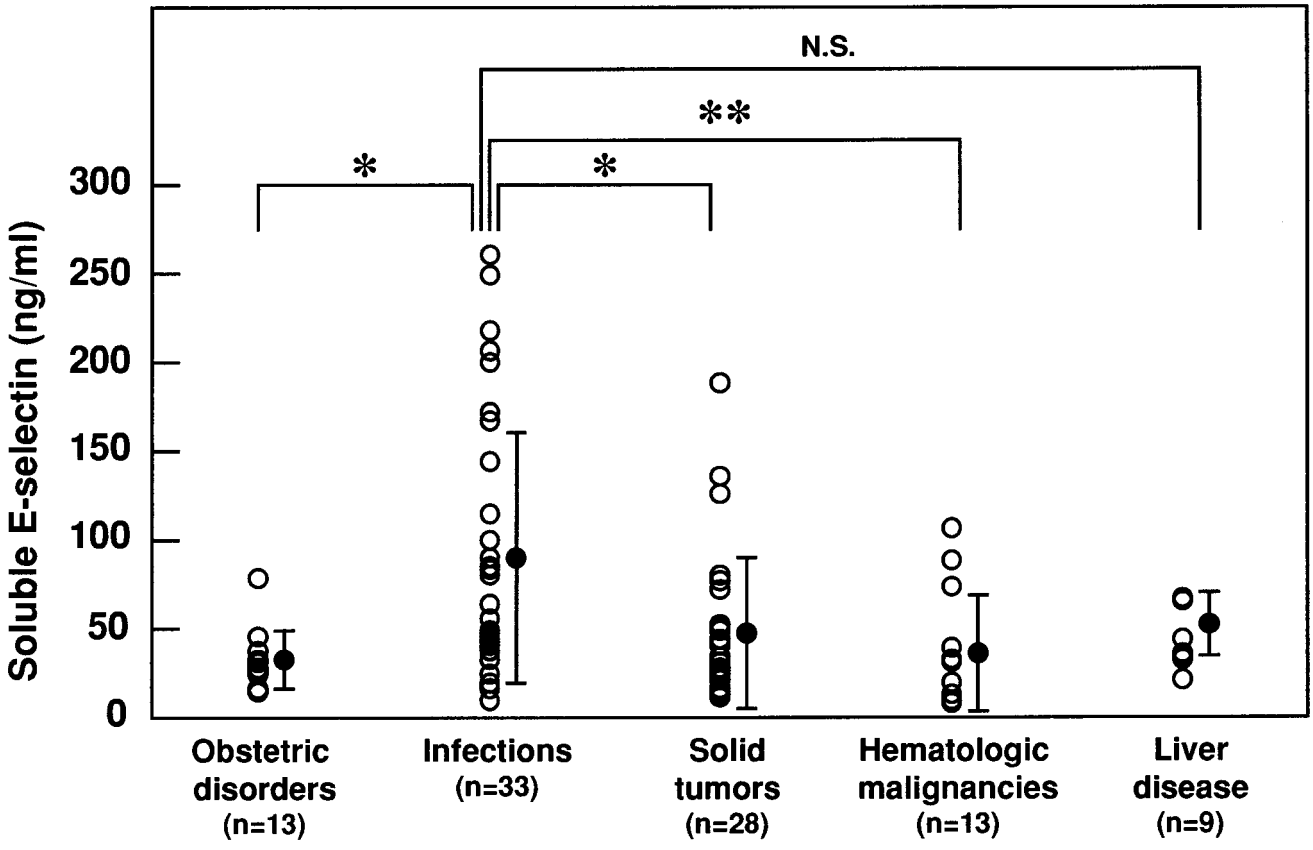

Fig. 1. Plasma levels of soluble E-selectin in 96 patients with suspected disseminated intravascular coagulation. *$P < 0.01$; **$P < 0.02$; NS, not significant.

tion, which leads to disseminated intravascular coagulation (DIC) in the presence of sepsis. However, serum levels of TNF-α or IL-1β have been detected in only a small number of patients with sepsis [9,10]. Therefore, the contribution of these cytokines to DIC in patients with sepsis remains to be determined.

We measured plasma levels of sE in patients with infections and other underlying diseases with and without DIC to clarify whether the plasma level of sE reflects cytokine-induced endothelial activation.

MATERIALS AND METHODS

We measured plasma levels of sE in 96 patients suspected of having DIC, including 33 patients with infections, 28 patients with solid tumors, 13 patients with obstetric disorders, 13 patients with hematological malignancies, and 9 patients with liver diseases. DIC was diagnosed in 63 of the 96 patients based on the following criteria [11]: 1) presence of an underlying disorder frequently associated with DIC, 2) positive soluble fibrin monomer complex (SFMC) and elevated FDP(E) levels (>500 ng/ml), and 3) presence of clinical bleeding or organ dysfunction.

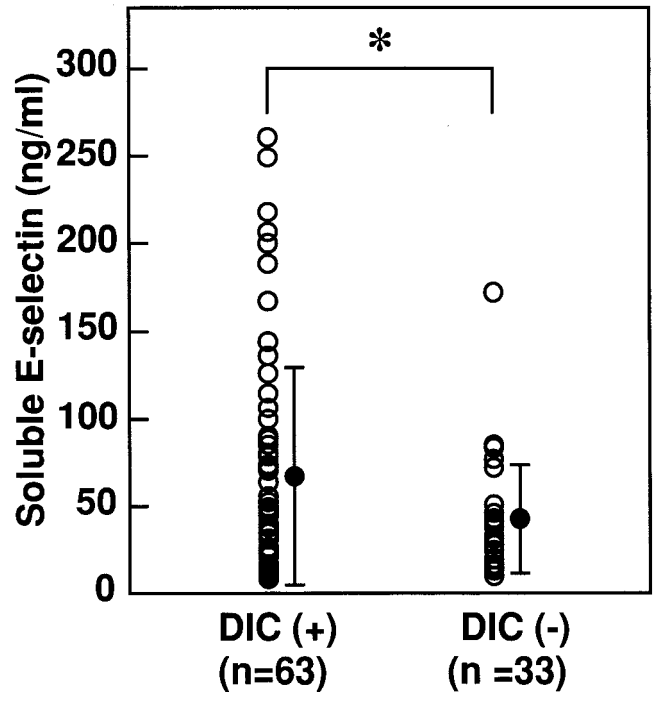

Fig. 2. Plasma levels of soluble E-selectin in 96 patients with (+) and without (−) disseminated intravascular coagulation. *$P < 0.02$.

TABLE I. Correlation of sE-Selectin With FDP (E) in Patients Suspected of Having DIC With Various Underlying Disorders*

		Underlying disorders				
	Whole patients (n = 96)	Obstetric disorders (n = 13)	Infections (n = 33)	Solid tumors (n = 28)	Hematologic malignancies (n = 13)	Liver disease (n = 9)
Correlation coefficient	0.29 ($P < 0.005$)	0.01 (NS)	0.51 ($P < 0.003$)	0.27 (NS)	0.27 (NS)	0.62 (NS)

*NS, not significant.

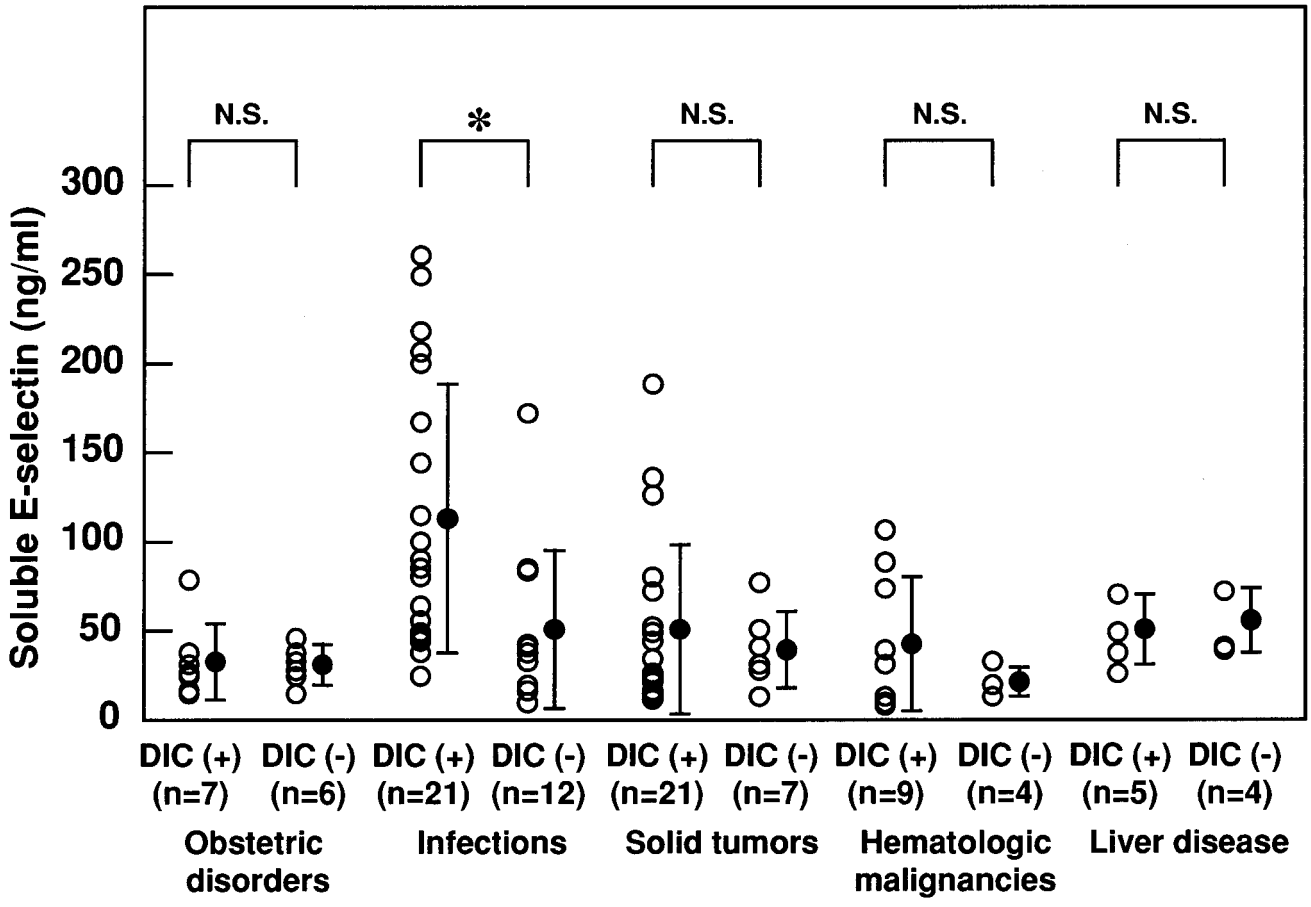

Fig. 3. Plasma levels of soluble E-selectin in patients with infection and other underlying disorders with (+) and without (−) disseminated intravascular coagulation. *$P < 0.02$.

Organ failure was defined according to the following criteria [12]: respiratory failure, patients who required mechanical ventilation; renal failure, urine output <500 ml/day, serum urea nitrogen >50 mg/dl, or serum creatinine >2 mg/dl; hepatic failure, total serum bilirubin >3 mg/dl or transaminases (AST and ALT) >100 U/ml; heart failure, cardiac arrest, or arrhythmia (atrial-ventricular block or evidence for acute myocardial infarction), central venous pressure >20 cm H_2O or need for vasoactive or inotropic agents; and gastrointestinal bleeding, hematemesis or melena requiring transfusion. Organ failure was identified in 41 patients.

Plasma Samples

Blood samples were obtained from patients in tubes containing a 1/10 volume of 3.8% sodium citrate and were centrifuged at 2,000*g* for 10 min at 4°C. The plasma level of soluble E-selectin (sE) was measured by an enzyme-linked immunosorbent assay (ELISA) method (Bender-Med, Vienna, Austria). The mean sE level in an age-matched population of 21 healthy human subjects was 25.9 ± 8.3 ng/ml (± SD). The level of SFMC was determined by the fibrin monomer-coated erythrocyte aggregation method [13]. The serum level of FDP(E) was measured by a latex aggregation assay, as previously

described [12]. The plasma level of TNF-α was determined using an ELISA kit for human TNF-α (Otsuka Pharmaceutical, Tokyo, Japan). The serum level of interleukin-6 (IL-6) was determined using an ELISA kit (Genzyme, Cambridge, MA).

Statistical Analysis

Data are mean ± SD. Data were analyzed using analysis of variance and Scheffe's post hoc test or unpaired t-test. $P < 0.05$ was accepted as statistically significant.

RESULTS

Plasma Levels of sE in Patients Suspected of Having DIC

The plasma level of sE was significantly higher in patients with infection (90.5 ± 71.2 ng/ml) than in patients with obstetric disorders (32.1 ± 16.8 ng/ml), solid tumors (47.2 ± 41.8 ng/ml), and hematologic malignancies (36.0 ± 32.8 ng/ml). Although the plasma level of sE tended to be higher in patients with infections than in patients with liver disease (53.3 ± 18.3 ng/ml), the difference was not significant (Fig. 1).

Relationship Between Plasma Levels of sE and DIC

The plasma level of sE was significantly higher in patients with DIC (68.0 ± 61.8 ng/ml) than in patients without DIC (42.0 ± 30.9 ng/ml) in the overall study population (Fig. 2).

The plasma level was significantly higher in the 21 patients with infection with DIC (112.7 ± 74.9 ng/ml) than in the 12 patients with infection without DIC (51.6 ± 44.4 ng/ml, $P < 0.02$) (Fig. 3), but there was no significant difference between patients with other underlying disorders with and without DIC. The plasma level of sE was significantly correlated with serum level of FDP(E) in patients with infection, but not in patients with underlying disorders other than infection (Table I).

Relationship Between the Plasma Level of sE and Organ Failure

The plasma level of sE was significantly higher in the 41 patients with organ failure (89.0 ± 64.9 ng/ml) than in the 55 patients without organ failure (36.8 ± 30.3 ng/ml) (Fig. 4). When the data were analyzed for subgroup of patients classified according to disease, only patients with infections with and without organ failure (118.9 ± 77.4 ng/ml and 46.7 ± 24.7 ng/ml, respectively) showed a significant difference in plasma levels of sE (Fig. 5).

Plasma Levels of TNF-α and IL-6 in 96 Patients

The plasma level of TNF-α was detected in only 12.1% and 7.6% of the patients with infections and solid tumors, respectively. Plasma levels of TNF-α were not detected in patients with the other underlying disorders. The plasma level of IL-6 was detected in 20.0% of patients with infections, 22.2% of those with liver diseases, 23.1% of those with hematologic malignancies, 23.1% of those with solid tumors, and 12.5% of those with obstetric disorders. No significant differences in plasma levels of IL-6 were found between those with and without DIC or organ dysfunction in 96 patients (data not shown).

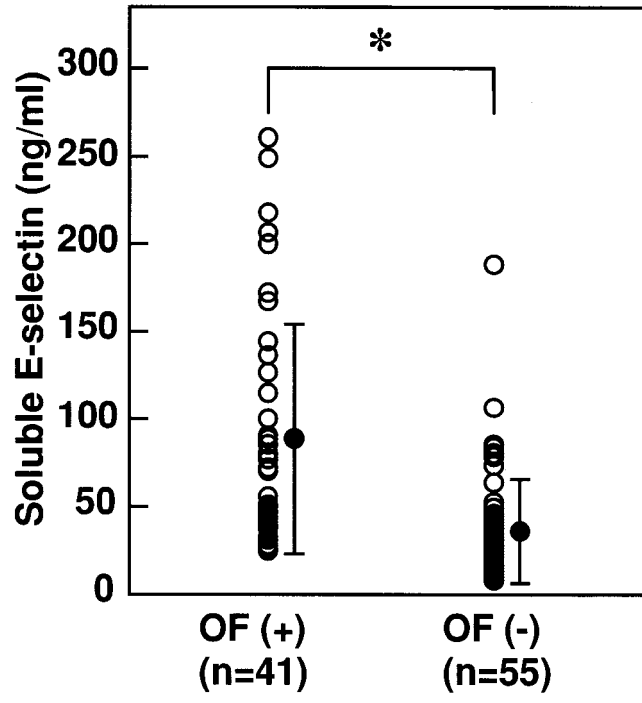

Fig. 4. Plasma levels of soluble E-selectin in 96 patients with (+) and without (−) organ failure. OF, organ failure. *$P < 0.01$.

DISCUSSION

The present study demonstrates that plasma levels of sE may reflect the activation of endothelial cells induced by cyotkines such as TNF-α and IL-1β. Plasma levels of sE were significantly elevated in patients with DIC compared with patients with infection without DIC, but there was no significant difference in plasma levels of sE between patients with other underlying disorders with and without DIC. The present findings are consistent with the results of a study by Drake et al. [14], who found that expression of E-selectin was elevated in the systemic microvasculature of baboons with lethal *E. coli* sepsis. Redl et al. [15] also reported that the expression of E-hypovolemic shock. We previously reported that monocytes may be important in the activation of intravascular coagulation in patients with septicemia [16].

The present findings also suggest that DIC may be caused by various mechanisms, depending on the nature of the underlying disorder. In patients with obstetric dis-

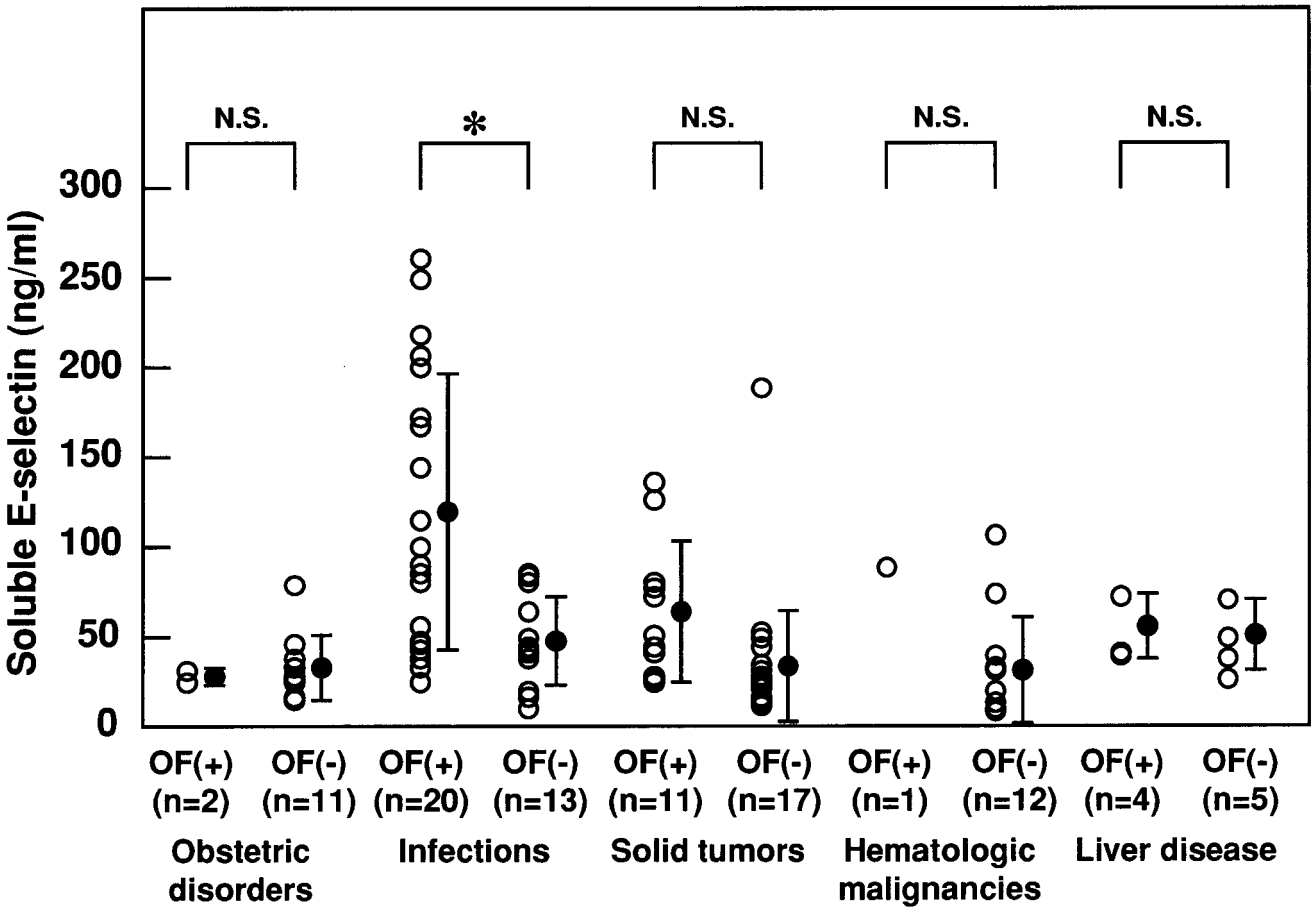

Fig. 5. Plasma levels of soluble E-selectin in patients with infection and other underlying disorders with (+) and without (−) organ failure. OF, organ failure. *$P < 0.01$; NS, not significant.

orders such as abruptio placenta, tissue factor derived from the necrotized placenta may contribute to activation of intravascular coagulation [17]. Wada et al. [18] reported that plasma levels of interleukin-1β were significantly elevated in DIC patients with sepsis, but not in DIC patients with acute leukemia, suggesting that the mechanism of DIC in patients with acute leukemia may differ from that in patients with septicemia. The present observations are consistent with their findings.

Although plasma levels of sE reflect the induction of endothelial activation by cytokines such as TNF-α and IL-1β, TNF-α and IL-1β have been detected in only 36.5% and 37.0%, respectively, of patients with sepsis [9,10]. In the present study, TNF-α was detected in only 4 (12.1%) of 33 patients with infections. It is not clear why TNF-α is detected in such a small number of patients with sepsis. If the sE level reflects endothelial activation induced by these cytokines, then determinations of sE instead of these cytokines may help clarify the mechanism of DIC.

Plasma levels of sE were related to the presence of organ failure in patients with infections in the present study. Organ failure may have been related to endothelial injury induced by the direct action of cytokines [19], or by the indirect actions of cytokine-activated neutrophils [20]. Thus, the plasma level of sE may be related to both DIC and organ failure in patients with infections and may identify patients at high risk for multiple organ failure. Plasma levels of cytokines, such as TNF-α, IL-1β, and IL-6 have been found to be correlated with survival in patients with sepsis [10], which is consistent with this hypothesis. Thus, determination of the plasma level of sE in patients with infections may be of prognostic value. We are currently investigating this possibility in a controlled prospective study.

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